WO9951743

Translation from Japanese of the Field of the Invention

Date of publication: 14.10 1999

Translated by NBT

The present invention relates to a human/mouse chimera antibody comprising a variable region (V region) of a mouse monoclonal antibody against human tissue factor (TF) and a constant region (C region) of a human antibody, a humanized antibody wherein a complementarity-determining region (CDR) of a light chain (L chain) V region and a heavy chain (H chain) V region of a mouse monoclonal antibody against human TF is grafted into a human antibody, an L chain and an H chain of the antibody, and a fragment of a V region which constitutes an L chain or an H chain of the antibody. The invention also relates to a process for constructing a humanized antibody against human TF.

The invention further relates to a DNA encoding the above-mentioned antibody, particularly a fragment of its V region, and a DNA encoding an L chain or H chain containing the V region. The invention also relates to a recombinant vector containing the DNA and a host transformed with the vector.

The invention further relates to a process for producing a chimera antibody against human TF and a humanized antibody. The invention also relates to a pharmaceutical composition containing a humanized antibody against human TF as an effective ingredient and a medicament for treating disseminated intravascular coagulation (DIC).

Translation of Claims

1. A chimera H chain comprising a heavy (H) chain variable (V) region of a mouse monoclonal antibody against human tissue factor (TF) and an H chain constant (C) region of a human antibody, wherein said H chain V region has any amino acid sequence of

(1) an amino acid sequence of SEQ ID NO:139 (ATR-2),

(2) an amino acid sequence of SEQ ID NO:140 (ATR-3),

(3) an amino acid sequence of SEQ ID NO:141 (ATR-4),

(4) an amino acid sequence of SEQ ID NO:142 (ATR-5),

(5) an amino acid sequence of SEQ ID NO:143 (ATR-7), and

(6) an amino acid sequence of SEQ ID NO:144 (ATR-8).

2. The chimera H chain according to claim 1, wherein said H chain V region has the amino acid sequence of SEQ ID NO:142.

3. The chimera H chain according to claim 1 or 2, wherein said H chain C region is a Cγ1, Cγ2, Cγ3 or Cγ4 region.

4. The chimera H chain according to any one of claims 1 to 3, wherein said H chain V region has the amino acid sequence of SEQ ID NO:142, and said H chain C region is Cγ4.

5. A chimera L chain comprising a light (L) chain V region of a mouse monoclonal antibody against human TF and an L chain C region of a human antibody, wherein said L chain V region has any amino acid sequence of:

(1) an amino acid sequence of SEQ ID NO:145 (ATR-2),

(2) an amino acid sequence of SEQ ID NO:146 (ATR-3),

(3) an amino acid sequence of SEQ ID NO:147 (ATR-4),

(4) an amino acid sequence of SEQ ID NO:148 (ATR-5),

(5) an amino acid sequence of SEQ ID NO:149 (ATR-7), and

(6) an amino acid sequence of SEQ ID NO:150 (ATR-8).

6. The chimera L chain according to claim 5, wherein said L chain V region has the amino acid sequence of SEQ ID NO:148.

7. The chimera L chain according to claim 5 or 6, wherein said L chain C region is a Cλ or Cκ region.

8. The chimera L chain according to any one of claims 5 to 7, wherein said L chain V region has the amino acid sequence of SEQ ID NO:148, and said L chain C region is Cκ.

9. A chimera antibody against human TF comprising the chimera H chain according to any one of claims 1 to 4 and the chimera L chain according to any one of claims 5 to 8.

10. A chimera antibody against human TF comprising the chimera H chain according to claim 4 and the chimera L chain according to claim 8.

11. A humanized H chain V region comprising a complementarity-determining region (CDR) of an H chain V region of a mouse monoclonal antibody against human TF and a framework region (FR) of an H chain V region of a human antibody, wherein said CDR has the following amino acid sequences:

H-CDR1: Asp Tyr Tyr Met His (SEQ ID NO:133)

H-CDR2: Gly Asn Asp Pro Ala Asn Gly His Ser Met Tyr Asp Pro Lys Phe Gln Gly (SEQ ID NO:134)

H-CDR3: Asp Ser Gly Tyr Ala Met Asp Tyr (SEQ ID NO:135).

12. A humanized H chain V region, wherein said FR has the following amino acid sequences:

H-FR1: Gln Val Gln Leu Leu Glu Ser Gly Ala Val Leu Ala Arg Pro Gly Thr Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys (SEQ ID NO:110)

H-FR2: any of the following sequences (1) to (3):

(1) Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp I1e Gly (SEQ ID NO:111)

(2) Try Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly (SEQ ID NO:112)

(3) Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly (SEQ ID NO:113)

H-FR3: any of the following sequences (1) to (10):

(1) Arg Ala Lys Leu Thr Ala Ala Thr Ser Ala Ser Ile Ala Tyr Leu Glu Phe Ser Ser Leu Thr Asn Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg (SEQ ID NO:114)

(2) Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asn Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Arg (SEQ ID NO:115)

(3) Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg (SEQ ID NO:116)

(4) Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg (SEQ ID NO:117)

(5) Arg Val Ser Ile Thr Ala Asp Glu Ser Thr Lys Ile Ala Tyr Met Glu Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg (SEQ ID NO:118)

(6) Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg (SEQ ID NO:119)

(7) Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg (SEQ ID NO:120)

(8) Arg Val Thr Met Ser Ala Asp Lys Ser Ser Ser Ala Ala Tyr Leu Gln Trp Thr Ser Leu Lys Ala Ser Asp Thr Ala Ile Tyr Phe Cys Ala Arg (SEQ ID NO:121)

(9) Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Val Phe Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg (SEQ ID NO:122)

(10) Arg Val Thr Phe Thr Ala Asp Thr Ser Ala Asn Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg (SEQ ID NO:123)

FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser (SEQ ID NO:124).

13. The humanized H chain V region according to claim 11 or 12, which has the amino acid sequence represented by SEQ ID NO:30 (version a), SEQ ID NO:40 (version b), SEQ ID NO:42 (version c), SEQ ID NO:50 (version d), SEQ ID NO:52 (version e), SEQ ID NO:58 (version f), SEQ ID NO:60 (version g), SEQ ID NO:64 (version h), SEQ ID NO:70 (version i), SEQ ID NO:72 (version j), SEQ ID NO:76 (version b1), SEQ ID NO:78 (version d1), SEQ ID NO:82 (version b3) or SEQ ID NO:84 (version d3).

14. The humanized H chain V region according to any one of claims 11 to 13, which has the amino acid sequence of SEQ ID NO:40 (version b).

15. The humanized H chain V region according to any one of claims 11 to 13, which has the amino acid sequence of SEQ ID NO:70 (version i).

16. A humanized L chain V region comprising a CDR of an L chain V region of a mouse monoclonal antibody against human TF and an FR of a human L chain V region, wherein said CDR has the following amino acid sequences:

L-CDR1: Lys Ala Ser Gln Asp Ile Lys Ser Phe Leu Ser (SEQ ID NO:136)

L-CDR2: Tyr Ala Thr Ser Leu Ala Asp (SEQ ID NO:137)

L-CDR3: Leu Gln His Gly Glu Ser Pro Tyr Thr (SEQ ID NO:138).

17. The humanized L chain V region according to claim 16, wherein said FR has the following amino acid sequences:

L-FR1: Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (SEQ ID NO:125)

L-FR2: any of the following sequences (1) to (3):

(1) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr (SEQ ID NO:126)

(2) Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Thr Leu Ile Tyr (SEQ ID NO:127)

(3) Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile Tyr (SEQ ID NO:128)

L-FR3: any of the following sequences (1) to (3):

(1) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (SEQ ID NO:129)

(2) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (SEQ ID NO:130)

(3) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys (SEQ ID NO:131)

L-FR4: Phe Gly Gly Gly Thr Lys Val Glu Ile Lys (SEQ ID NO:132).

18. The humanized L chain V region according to claim 16 or 17, which has the amino acid sequence represented by SEQ ID NO:93 (version a), SEQ ID NO:99 (version b), SEQ ID NO:101 (version c), SEQ ID NO:107 (version b1) or SEQ ID NO:109 (version b2).

19. The humanized L chain V region according to any one of claims 16 to 18, which has the amino acid sequence of SEQ ID NO:99 (version b).

20. The humanized L chain V region according to any one of claims 16 to 18, which has the amino acid sequence of SEQ ID NO:109 (version b2).

21. A humanized H chain of an antibody against human TF, comprising the humanized H chain V region according to any one of claims 11 to 15 and an H chain C region of a human antibody.

22. A humanized H chain of an antibody against human TF, comprising the humanized H chain V region (version b) according to claim 14 and an H chain C region of a human antibody.

23. A humanized H chain of an antibody against human TF, comprising the humanized H chain V region (version i) according to claim 15 and an H chain C region of a human antibody.

24. The humanized H chain according to any one of claims 21 to 23, wherein said H chain C region of a human antibody is Cγ1, Cγ2, Cγ3 or Cγ4.

25. A humanized L chain of an antibody against human TF, comprising the humanized L chain V region according to any one of claims 16 to 20 and an L chain C region of a human antibody.

26. A humanized L chain of an antibody against human TF, comprising the humanized L chain V region (version b) according to claim 19 and an L chain C region of a human antibody.

27. A humanized L chain of an antibody against human TF, comprising the humanized L chain V region (version b2) according to claim 20 and an L chain C region of a human antibody.

28. The humanized L chain according to any one of claims 25 to 27, wherein said L chain C region of a human antibody is Cλ or Cκ.

29. A humanized antibody against human TF, comprising the humanized H chain according to any one of claims 21 to 24 and the humanized L chain according to any one of claims 25 to 28.

30. A humanized antibody against human TF, comprising the humanized H chain (version b) according to claim 22 and the humanized L chain (version b) according to claim 26.

31. A humanized antibody against human TF, comprising the humanized H chain (version i) according to claim 23 and the humanized L chain (version b) according to claim 26.

32. A humanized antibody against human TF, comprising the humanized H chain (version i) according to claim 23 and the humanized L chain (version b2) according to claim 27.

33. A DNA encoding the chimera H chain according to any one of claims 1 to 4.

34. A DNA encoding the chimera H chain according to any one of claims 2 to 4.

35. A DNA encoding the chimera L chain according to any one of claims 5 to 8.

36. A DNA encoding the chimera L chain according to any one of claims 6 to 8.

37. A DNA encoding the humanized H chain V region according to any one of claims 11 to 15.

38. A DNA encoding the humanized H chain V region (version b) according to claim 14.

39. A DNA encoding the humanized H chain V region (version i) according to claim 15.

40. A DNA encoding the humanized L chain V region according to any one of claims 16 to 20.

41. A DNA encoding the humanized L chain V region (version b) according to claim 19.

42. A DNA encoding the humanized L chain V region (version b2) according to claim 20.

43. A DNA encoding the humanized H chain according to any one of claims 21 to 24.

44. A DNA encoding the humanized H chain (version b) according to claim 22 or 24.

45. A DNA encoding the humanized H chain (version i) according to claim 23 or 24.

46. A DNA encoding the humanized L chain according to any one of claims 25 to 28.

47. A DNA encoding the humanized L chain (version b) according to claim 26 or 28.

48. A DNA encoding the humanized L chain (version b2) according to claim 27 or 28.

49. An expression vector comprising the DNA encoding the chimera H chain according to claim 33.

50. An expression vector comprising the DNA encoding the chimera H chain according to claim 34.

51. An expression vector comprising the DNA encoding the chimera L chain according to claim 35.

52. An expression vector comprising the DNA encoding the chimera L chain according to claim 36.

53. An expression vector comprising the DNA encoding the chimera H chain according to claim 33 and the DNA encoding the chimera L chain according to claim 35.

54. An expression vector comprising the DNA encoding the chimera H chain according to claim 34 and the DNA encoding the chimera L chain according to claim 36.

55. An expression vector comprising the DNA encoding the humanized H chain according to claim 43.

56. An expression vector comprising the DNA encoding the humanized H chain (version b) according to claim 44.

57. An expression vector comprising the DNA encoding the humanized H chain (version i) according to claim 45.

58. An expression vector comprising the DNA encoding the humanized L chain according to claim 46.

59. An expression vector comprising the DNA encoding the humanized L chain (version b) according to claim 47.

60. An expression vector comprising the DNA encoding the humanized L chain (version b2) according to claim 48.

61. An expression vector comprising the DNA encoding the humanized H chain according to claim 43 and the DNA encoding the humanized L chain according to claim 46.

62. An expression vector comprising the DNA encoding the humanized H chain (version b) according to claim 44 and the DNA encoding the humanized L chain (version h) according to claim 47.

63. An expression vector comprising the DNA encoding the humanized H chain (version i) according to claim 45 and the DNA encoding the humanized L chain (version b) according to claim 47.

64. An expression vector comprising the DNA encoding the humanized H chain (version i) according to claim 45 and the DNA encoding the humanized L chain (version b2) according to claim 48.

65. A host transformed with the expression vector comprising the DNA encoding the chimera H chain according to claim 49 and the expression vector comprising the DNA encoding the chimera L chain according to claim 51.

66. A host transformed with the expression vector comprising the DNA encoding the chimera H chain according to claim 50 and the expression vector comprising the DNA encoding the chimera L chain according to claim 52.

67. A host transformed with the expression vector according to claim 53.

68. A host transformed with the expression vector according to claim 54.

69. A host transformed with the expression vector comprising the DNA encoding the humanized H chain according to claim 55 and the expression vector comprising the DNA encoding the humanized L chain according to claim 58.

70. A host transformed with the expression vector comprising the DNA encoding the humanized H chain (version b) according to claim 56 and the expression vector comprising the DNA encoding the humanized L chain (version b) according to claim 59.

71. A host transformed with the expression vector comprising the DNA encoding the humanized H chain (version i) according to claim 57 and the expression vector comprising the DNA encoding the humanized L chain (version b) according to claim 59.

72. A host transformed with the expression vector comprising the DNA encoding the humanized H chain (version i) according to claim 57 and the expression vector comprising the DNA encoding the humanized L chain (version b2) according to claim 6.

73. A host transformed with the expression vector according to claim 61.

74. A host transformed with the expression vector according to claim 62.

75. A host transformed with the expression vector according to claim 63.

76. A host transformed with the expression vector according to claim 64.

77. A process for producing a chimera antibody against human TF, which comprises culturing the host according to claim 65 and collecting a chimera antibody from said culture product.

78. A process for producing a chimera antibody against human TF, which comprises culturing the host according to claim 66 and collecting a chimera antibody from said culture product.

79. A process for producing a chimera antibody against human TF, which comprises culturing the host according to claim 67 and collecting a chimera antibody from said culture product.

80. A process for producing a chimera antibody against human TF, which comprises culturing the host according to claim 68 and collecting a chimera antibody from said culture product.

81. A process for producing a humanized antibody against human TF, which comprises culturing the host according to claim 69 and collecting a humanized antibody from said culture product.

82. A process for producing a humanized antibody against human TF, which comprises culturing the host according to claim 70 and collecting a humanized antibody from said culture product.

83. A process for producing a humanized antibody against human TF, which comprises culturing the host according to claim 71 and collecting a humanized antibody from said culture product.

84. A process for producing a humanized antibody against human TF, which comprises culturing the host according to claim 72 and collecting a humanized antibody from said culture product.

85. A process for producing a humanized antibody against human TF, which comprises culturing the host according to claim 73 and collecting a humanized antibody from said culture product.

86. A process for producing a humanized antibody against human TF, which comprises culturing the host according to claim 74 and collecting a humanized antibody from said culture product.

87. A process for producing a humanized antibody against human TF, which comprises culturing the host according to claim 75 and collecting a humanized antibody from said culture product.

88. A process for producing a humanized antibody against human TF, which comprises culturing the host according to claim 76 and collecting a humanized antibody from said culture product.

89. A process for producing a natural humanized antibody having a complementarity-determining region (CDR) derived from a non-human origin and a framework region (FR) derived from a natural human antibody and possessing reduced immunogenicity, which comprises:

(1) preparing a non-human monoclonal antibody reactive to an aimed antigen,

(2) preparing plural human antibodies having a high homology to the amino acid sequence of FR in the monoclonal antibody of said (1),

(3) constructing a first humanized antibody by substituting four FRs of one kind of the human antibody in said (2) with corresponding FRs of the non-human monoclonal antibody said (1),

(4) measuring a binding capacity of the humanized antibody constructed in said (3) toward the antigen or an ability of the antibody to neutralize the biological activity of the antigen,

(5) constructing a second humanized antibody by substituting one to three FRs of the humanized antibody constructed in said (3) with corresponding FRs of the human antibody different from that used in (3) among the human antibodies prepared in (2),

(6) comparing the second humanized antibody constructed in said (5) and the first humanized antibody obtained in said (3) in view of the binding capacity toward the antigen or the ability to neutralize the biological activity of the antigen, and selecting a humanized antibody exhibiting a preferable activity,

(7) carrying out the steps of said (3) to (6) upon the humanized antibody selected in said (6), and

(8) repeating the steps of said (3) to (6) until a humanized antibody having an activity equal to that of the non-human monoclonal antibody in said (1).

90. The process according to claim 89, wherein said aimed antigen is a human tissue factor (TF).

91. A humanized antibody obtainable through the process of claim 89.

92. A humanized antibody obtainable through the process of claim 90.

93. A medicament for treating disseminated intravascular coagulation (DIC) comprising the humanized antigen according to any one of claims 29 to 32 and 92.